other types of liquids. Of particular interest are biological fluids such as serum, plasma, urine, cerebrospinal fluid, saliva, milk, broth and other culture media and supernatants as well as fractions of any of them. Other sources of sample fluid which are tested by conventional methods are contemplated as within the meaning of this term as used and can, likewise, be assayed in accordance with the invention.

The term "ligand" refers to any substance, or class of related substances, whose presence is to be qualitatively or quantitatively determined in a sample fluid, such as those just described. The present assay can be applied to the detection of ligands for which there is a specific binding partner and, conversely, to the detection of the capacity of a liquid medium to bind a ligand (usually due to the presence of a binding partner for the ligand in the sample). The ligand usually is a peptide, protein, carbohydrate, glycoprotein, steroid, or other organic molecule for which a specific binding partner exists or can be provided by immunological or synthetic means. The ligand, in functional terms, is usually selected from antigens and antibodies thereto; haptens and antibodies thereto; and hormones, vitamins, metabolites and pharmacological agents and their receptors and binding substances and normal serum constituents and disease markers.

The terms "binding partner" or "receptor" refer to any substance, or class of substances, which has a specific binding affinity for the ligand in preference to other substances. In the majority of embodiments, the present invention will incorporate specific binding assay reagents which interact with the ligand or its binding effectors in the sample in an immunochemical manner. That is, there will be an antigen-antibody or hapten-antibody relationship between reagents and/or the ligand or its binding effector in the sample. Such assays therefore are termed immunoassays and the special interaction between the ligand and its receptor, or binding partner, is immonochemical binding. However, it is well understood in the art that other binding interactions between the ligand and the binding partner serve as the basis of specific binding assays, including the binding interactions between hormones, vitamins, metabolites, and pharmacological agents, and their respective receptors and binding substances. For example, polypeptide hormone receptors as binding agents or partners are discussed in Langan, et al., (Eds.), *Ligand Assay,* Masson Publishing U.S.A. Inc., New York, pages 211 et seq (1981).

The term "selectively accessible vesicle" refers to single or multi-compartmented sacs enclosing an internal volume, having a wall composed of one or more components and forming one or more internal compartments which constitute the internal volume. One example of such a vesicle is a cell ghost, formed by opening a cellular membrane, removing the internal components of the cell and resealing the membrane. Another example is a liposome, a single or multicompartmented vesicle comprised of lipids, particularly lipid mixtures including at least one phospholipid, which form a continuous wall or bilayer lipid membrane. Additional common constituents of these lipid mixtures are cholesterol and charged long chain phospholipids. Liposomes can be prepared by any of a number of techniques. For example, multilamellar vesicles (MLVs) can be prepared by film evaporation and hydration of the lipid film. Reverse phase evaporation vesicles (REVs) may also be prepared. These are exemplary of techniques providing useful vesicles. For a general overview of liposomes and their formation, see Papahadjopoulos, et al., (Eds), Liposomes, Ann. N.Y. Acad. Sci., volume 308 (1978); Tom, et al., (Eds.), *Liposomes and Immunobiology,* Elsevier North Holland Inc., N.Y. (1980); and Gregoriadis, et al., *Liposomes in Biological Systems,* John Wiley & Sons, N.Y. (1980).

Liposomes can be made to have surface-incorporated ligand or ligand analog moieties. Such liposomes are formed using ligand-amphiphile conjugates, which usually take the form of a ligand-coupler-ampiphile molecule. Ampiphiles are substances which contain both water soluble and water insoluble regions. They are best exemplified by the lipid ampiphiles, such as the phosphatidyl ethanolamines, phosphatidyl serine, phosphatidyl inositol, sphingomyelin cerebrosides, phosphatidic acid, plasmalogens, cardiolipins and fatty acids.

Alternatively, ligands may be covalently bonded or adsorbed to the surface of preformed liposomes. When liposomes are preformed, they can have at their external surface several chemical functionalities to which antigens may be covalently linked. Appropriate reactions which may be applied to such couplings are described in Williams et al., *Methods in Immunology and Immunochemistry* Vol. 1, Academic Press, New York (1967). In some cases, antigens may be adsorbed to the liposome surface, as was shown by Uemura and Kinsky, Biochemistry, 11: 4085–4094 (1972).

The composition of the invention further includes a substance which modifies vesicle accessibility in response to binding of surface-incorporated ligand or ligand analog and the binding partner. The principal example of this substance is a group of compounds collectively referred to as complement. For a general overview of complement and its effects, see Rapp, et al., *Molecular Basis of Complement Action,* Appleton-Century-Crofts (1970). Also, the role of complement is discussed in many of the references addressing other liposome immunoassays which have been cited above.

The composition can use any of the variety of detection systems which have been recognized for such purposes, including those described in the references cited above. Additionally, the compositions can provide a detection system such as that described in co-pending Ser. No. 528,496 which was filed on Sept. 1, 1983 and is assigned to the instant assignee.

In accordance with the present invention, the composition further includes at least one surfactant which does not modify vesicle accessibility or interfere with the immunological interactions which form the basis for the specific binding assay. Such surfactants are included in the overall immunoassay reagent composition in a concentration range of from about 0.1 to about 1.0 percent.

One embodiment of the invention includes polyoxyethylene polymers having at least about 23 ethylene oxide monomer units. These can have, in addition, hydrophobic groups of from about C_8–C_{17}, including aromatic and aliphatic constituents. Examples of this embodiment include: polyoxyethylene lauryl ether having at least 23 ethylene oxide units (Brij-35, ICI United States, Inc., Wilmington, DE); nonyl phenoxy polyethoxyethanol having at least 30 ethylene oxide monomer units (Igepal CO-880, GAF Corp., N.Y., NY); and octyl phenoxy polyethoxy ethanol having at least 30 ethylene oxide monomer units (TRITON X-305, Rohm & Haas, Philadelphia PA).

Another embodiment of the invention includes surfactants which have the formula: